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Chapter

Ruthenium-Tris-Bipyridine Derivatives as a Divine Complex for Electrochemiluminescence Based Biosensor Applications

Chikkili Venkateswara Raju, Mathavan Sornambigai and Shanmugam Senthil Kumar

Abstract

In electrochemiluminescence (ECL) studies, Tris (bipyridine)ruthenium(II) chloride ($Ru(bpy)_3^{2+}$) and its derivatives have been used as primary luminophores since 1972. The flexible solubility in both aqueous and non-aqueous medium and the remarkable intrinsic properties like chemical, optical and desirable electrochemical behavior drives the researcher to use $Ru(bpy)_3^{2+}$ and its derivatives as highly active ECL probes in modern analytical science. Novel surface modification of $Ru(bpy)_3^{2+}$ based ECL platforms are highly useful in the selective and sensitive detection of biomolecules, DNA analysis, immunoassays detection, and imaging of the biologically important molecules in cells and tissue of living organisms. This chapter discusses and highlights the most significant works in $Ru(bpy)_3^{2+}$ based ECL properties of reaction mechanisms and their applications.

Keywords: electrochemiluminescence, $Ru(bpy)_3^{2+}$, biosensor, annihilation mechanism, co-reactant mechanism

1. Introduction

The Electrochemiluminescence (ECL) is a process where the emission of light occurs by an excited luminophore molecule generated by reactive intermediates at the interface of the electrode and electrolyte [1]. It involves in three different kinds of processes. The first process is an electrical step, where the reactive intermediates of luminophores are generated at the electrode-electrolyte interface during the scanning of potential or applying a constant potential. In the second step, an energetic electron transfer occurs between the reactive intermediate which leads to the formation of an excited luminophore. Then the third step is a luminescence process, where the excited luminophore emits light during relaxation to the ground state. The first ECL reaction was observed by David M. Hercules in 1964, which deals with the ECL of Rubrene molecule in a non-aqueous medium [2].

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1.1 ECL principle

In general, the ECL principle involves the conversion of electrical energy into radiative energy through a chemical reaction [1]. The energy required to produce an exciting luminophore molecule by the electrochemical method is referred to as a change of enthalpy which is denoted as ΔH [3]. The enthalpy change of a particular ECL reaction can be calculated by using the following equation.

$$-\Delta H\left(\text{in } eV\right) = \mathrm{E}_0{}^{oxi} - \mathrm{E}_0{}^{red} - 0.16\,eV \tag{1}$$

-ΔH refers enthalpy change, and E_0^{oxi} is oxidation potential, E_0^{red} is the reduction potential of the luminophore or co-reactant molecules. The numerical value of 0.16 eV is entropy factor (TΔS).

The energy required to generate first singlet excited state is determined by following equation.

$$\mathrm{E}_s\left(\text{in } eV\right) = 1239.8/\lambda\left(nm\right) \tag{2}$$

If, -ΔH≤Es, then the ECL system is referred as energy deficiency system; for energy sufficient system, -ΔH≥ Es.

1.2 Reaction mechanisms

Normally, ECL reactions follow only two kinds of reaction mechanisms named as annihilation mechanism and co-reactant mechanism. The reaction mechanism of any ECL system depends on the reaction conditions such as the selection of luminophore, potential sweep direction, and nature of electrolyte.

1.2.1 Annihilation mechanism

In the annihilation mechanism, only luminophore molecule alone will participate in the emission of light. The luminophore get oxidizes at anode during the positive potential sweep direction (anode direction) to generate cationic intermediate or cationic radical intermediate. At the same time, the anionic radical intermediate of luminophore molecule was generated at the cathode during the potential sweep towards cathode direction. Then an energetic electron transfer occurs between highly energetic anionic and cationic reactive radicals, leads to produce one excited and one ground state luminophore molecule. The excited luminophore molecule comes to the ground state by emitting energy in the form of photons. For example, the emission of light by $Ru(bpy)_3^{2+}$ molecule in acetonitrile with tetrabutylammonium tetrafluoroborate ($TBABF_4$) as an electrolyte is the best example of annihilation mechanism [4]. The reaction mechanism is given below.

$$Ru\left(bpy\right)_3^{2+} \rightarrow Ru\left(bpy\right)_3^{3+} + e^- \left(\text{oxidation } at \text{ anode}\right)\ \mathrm{E}_0 = 1.2\ \mathrm{V}\ vs\ SCE \tag{3}$$

$$Ru\left(bpy\right)_3^{2+} + e^- \rightarrow Ru\left(bpy\right)_3^{1+} \left(\text{reduction } at \text{ cathode}\right)\quad \mathrm{E}_0 = -1.4\ \mathrm{V}\ vs\ SCE \tag{4}$$

$$Ru\left(bpy\right)_3{}^{1+} + Ru\left(bpy\right)_3{}^{3+} \rightarrow Ru\left(bpy\right)_3{}^{2+} + Ru\left(bpy\right)_3{}^{2+*} \left(\text{excitation}\right) \tag{5}$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu\,(\text{emission}) \tag{6}$$

For the sake of better understanding the above mechanism is shown in **Figure 1**. The annihilation mechanism occurs only in organic electrolytes and it requires a wider potential window in order to obtain the ECL. Because of the gas evolution reactions (oxygen and hydrogen evolution), the annihilation mechanism is not taking place in an aqueous medium.

1.2.2 Co-reactant mechanism

The drawbacks of the annihilation mechanism are overcome by adding an additional reagent called as a co-reactant along with the luminophore, and the mechanism is called a co-reactant mechanism. In this type of mechanism two reagents were taken into the account, one is luminophore and the other is a co-reactant molecule. Further, the narrow potential window is sufficient to gain the ECL and it is applicable in both organic and aqueous electrolytic medium. Based on the potential sweep direction the co-reactant mechanism is further classified into two types which are discussed below.

1.2.2.1 Oxidative-reduction mechanism

The potential window is fixed to only the anodic region. The co-reactant get oxides first at the anode to form oxidizing radical intermediate which has the high

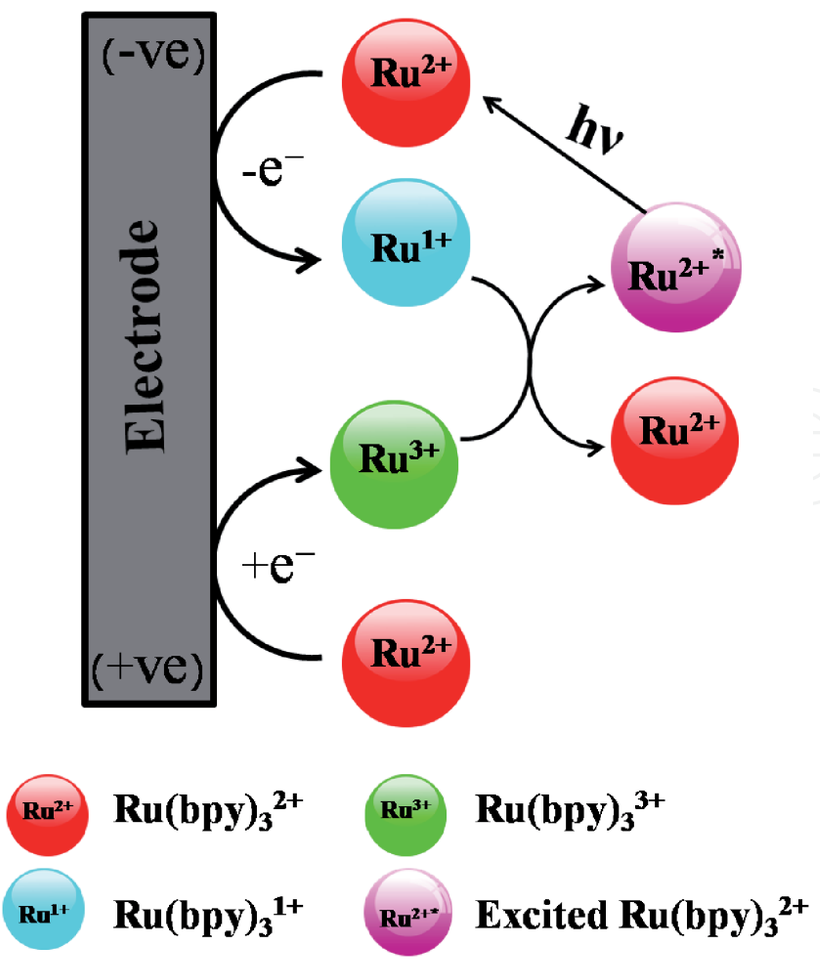

Figure 1.
The schematic representation of annihilation mechanism of $Ru(bpy)_3^{2+}$ molecule in acetonitrile.

reducing ability, then luminophore oxidizes to produce cationic reactive intermediate. After that, the co-reactant intermediate reduces the luminophore intermediate to generate an excited luminophore which emits light during energetic electron transfer reaction. The ECL of $Ru(bpy)_3^{2+}$ and tri-n-propyl amine (TPrA) system is the best example of an oxidative-reduction mechanism [5]. The reaction mechanism of $Ru(bpy)_3^{2+}$/TPrA system is given below.

$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + e^- \quad (7)$$

$$TPrA \rightarrow TPrA^{\bullet +} + e^- \quad (8)$$

$$TPrA^{\bullet +} \rightarrow TPrA^{\bullet} + H^+ \quad (9)$$

$$Ru(bpy)_3^{3+} + TPrA^{\bullet} \rightarrow Ru(bpy)_3^{2+*} + pr_2N^+CH = CH - CH_2 \quad (10)$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + \hbar \quad (11)$$

Here, $Ru(bpy)_3^{2+}$ is luminophore and TPrA acts as co-reactant. The schematic illustration for above reaction mechanism is also shown as **Figure 2.**

1.2.2.2 Reductive-oxidation mechanism

In this mechanism, the ECL is obtained by sweeping the potential exclusively to the cathode direction. The cathodic co-reactant gets reduced during cathodic potential scan where it produced high oxidizing ability of radical intermediate and then

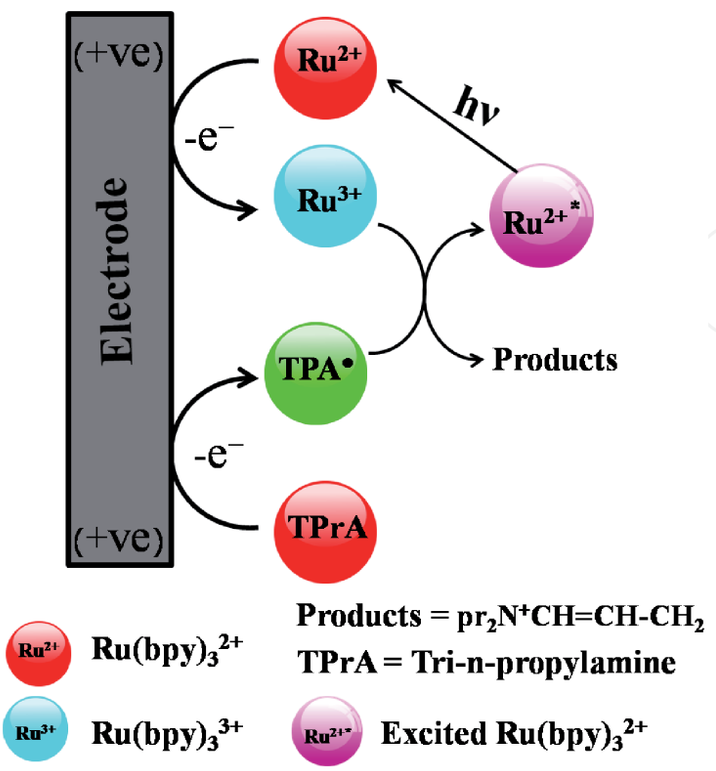

Figure 2.
The schematic illustration of oxidative-reduction mechanism of $Ru(bpy)_3^{2+}$ molecule and TPrA.

the followed by luminophores reduces to produce the anionic reactive intermediates. After that, the reduced radical intermediate of co-reactant oxidizes the anionic intermediate of the luminophore to form an excited luminophore which finally emits light. One of the classical examples for the reductive-oxidation mechanism is the ECL of $Ru(bpy)_3^{2+}$/per-sulphate ($S_2O_8^{2-}$) [6]. The reaction mechanism of this system is shown below.

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{\bullet -} + SO_4^{2-} \tag{12}$$

$$Ru(bpy)_3^{2+} + e^- \rightarrow Ru(bpy)_3^{1+} \tag{13}$$

$$Ru(bpy)_3^{1+} + SO_4^{\bullet -} \rightarrow Ru(bpy)_3^{2+*} + SO_4^{2-} \tag{14}$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu \tag{15}$$

Here, $Ru(bpy)_3^{2+}$ is luminophore and $S_2O_8^{2-}$ acts as co-reactant. The above mechanism is also given as schematic diagram which is indicated as **Figure 3**.

1.3 Role of $Ru(bpy)_3^{2+}$ and its derivatives as a luminophores in ECL

The first ECL experiment with $Ru(bpy)_3^{2+}$ as a luminophore was performed by A.J. Bard *et al.* in 1972 [4]. This discovery brought brightness to the ECL and

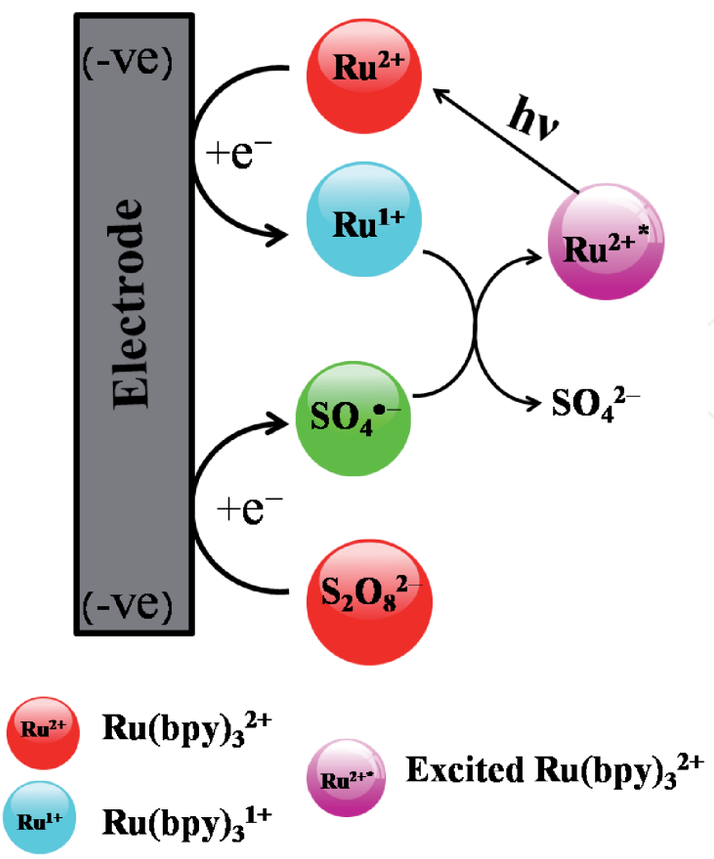

Figure 3.
The scheme of reductive-oxidation mechanism of $Ru(bpy)_3^{2+}$ molecule and persulfate.

created an endless platform for researchers to study the various kinds of ECL reactions. However, the ECL of $Ru(bpy)_3^{2+}$ was limited to organic electrolytes because the ECL follows annihilation mechanism which requires wide range potential window. To overcome this problem the ECL reaction within the narrow potential window was performed by taking additional reagent along with $Ru(bpy)_3^{2+}$ molecule. The first luminophore-co-reactant ECL reaction was carried out in 1981 by A.J. Bard group [7], oxalate was used as a first co-reactant to study the ECL reaction of $Ru(bpy)_3^{2+}$ molecule. Later on, various types of co-reactants were discovered like tri-n-propyl amine (TPrA), triethylamine (TEA), diethylamine (DEA), NADH, ascorbic acid, 2-(dibutyl amino) ethanol (DBAE), per-sulphates, hydrogen peroxide and glutathione etc [8]. $Ru(bpy)_3^{2+}$/co-reactant based ECL system plays a key role in a variety of analytical and clinical diagnostic applications. Recently *in-situ* generated co-reactants such as sulphate anion radicals and hydroxyl radicals also used as a new class of co-reactants to study the ECL of $Ru(bpy)_3^{2+}$ molecule in the aqueous system by using boron-doped diamond electrode (BDD) [9]. The superior ECL luminophore activity of $Ru(bpy)_3^{2+}$ molecule over the other luminophores is due to the high luminescent properties, elevated solubility in both organic and aqueous medium at room temperature, the reversible redox properties at the relevant potential region and high ECL quantum efficiency [10]. In general, $Ru(bpy)_3^{2+}$ has d^6 electronic configuration with octahedral structure, the emission of light is due to the metal to ligand charge transfer (MLCT) transition. The emission wavelength of $Ru(bpy)_3^{2+}$ lies between 600 to 650 nm.

In addition, the derivatives of $Ru(bpy)_3^{2+}$ were also shown their own contribution to ECL as luminophores. The linkage of aliphatic acids or aldehydes to the $Ru(bpy)_3^{2+}$ molecule gives different kind of luminophores called as acrylates. The ECL emission wavelength of $Ru(bpy)_3^{2+}$ was tuned by linking the different aliphatic compounds in acrylates (640 nm to 700 nm). And also, the $Ru(bpy)_3^{2+}$ conjugated with Schiff bases shown self-enhanced ECL signal with more intense light than $Ru(bpy)_3^{2+}$ molecule [11]. The enhanced ECL signal intensity is due to the resonance structure of imino radicals and presence of phenolic hydroxyl groups. Further, $Ru(bpy)_3^{2+}$ and its derivatives like $Ru(bpy)_3^{2+}$ dendrimers, and polypyridyl Ru-complexes used as a luminophore to study the bipolar ECL, microfluidic based ECL, wireless ECL [12, 13]. Apart from this, immobilization of $Ru(bpy)_3^{2+}$ molecule on polymer-coated electrodes shown new trend and remarkable ECL behaviour and created a solid-state platform for various kinds of analytical applications. In this context, $Ru(bpy)_3^{2+}$ incorporated on Nafion coated graphite electrode shows unusual ECL behaviour than solution-phase ECL system [14]. In similar way, $Ru(bpy)_3^{2+}$ on Nafion coated glassy carbon electrode (GCE) shown three ECL signals in co-reactant free oxygen saturated phosphate buffer solution (PBS).

Because of the excellent ECL behaviour exhibition by $Ru(bpy)_3^{2+}$ molecule, researchers tuned intrinsic properties of the $Ru(bpy)_3^{2+}$ molecule by introducing different functional groups into the parent $Ru(bpy)_3^{2+}$ and have been used in different analytical applications. In particular, $Ru(bpy)_3^{2+}$ utilized as an ECL probe in the detection of immunoassays [15], for example, the methylcytosine which belongs to a class of immunoassay detected by using ECL sensing method [13]. The DNA detection by ECL method has carried out by using $Ru(bpy)_3^{2+}$ molecule as ECL active material [16]. The double-standard DNA was detected by label-free ECL method using $Ru(phen)_3^{2+}$ as an ECL luminophore [17]. Apart from this, the ECL of $Ru(bpy)_3^{2+}$ also used in metal ions detection, bio-imaging, aptamer detection and other intracellular studies [13]. The schematic diagram is shown (**Figure 4**) the overall $Ru(bpy)_3^{2+}$ based ECL applications.

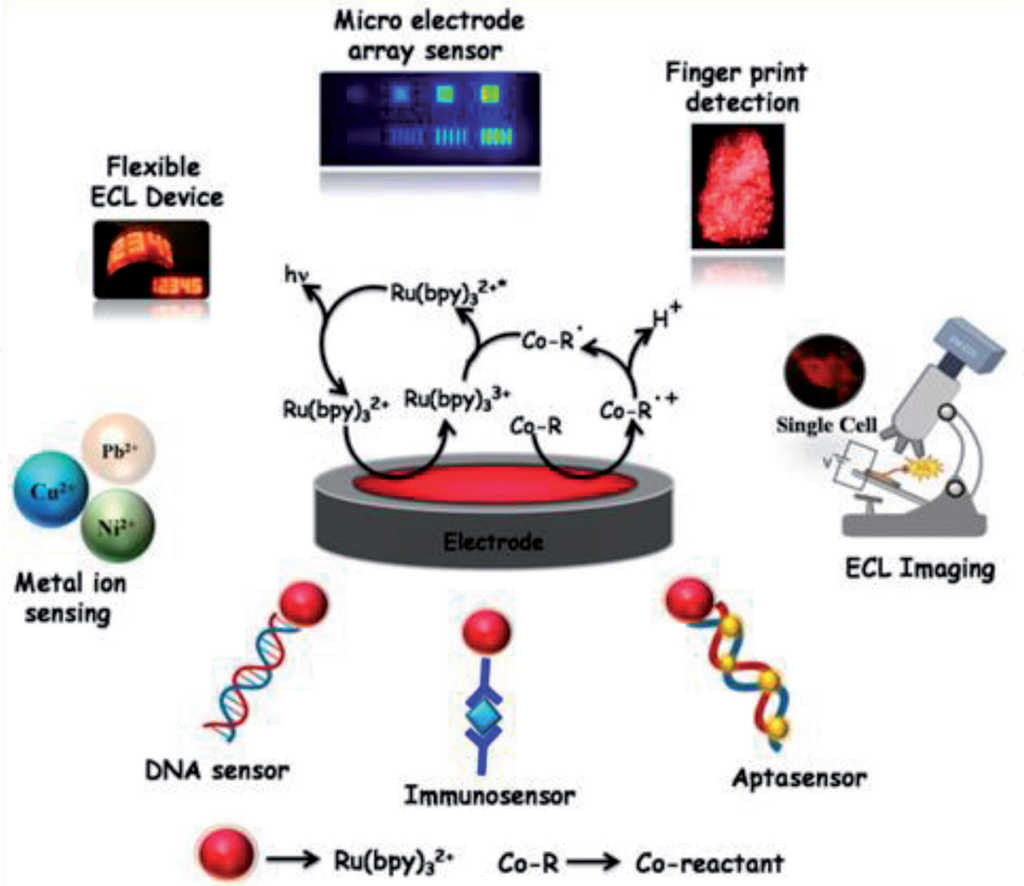

Figure 4.
Schematic illustration of ECL based applications using $Ru(bpy)_3^{2+}$ as a active probe.

2. Specific examples of $Ru(bpy)_3^{2+}$ and its derivatives for ECL studies

The ECL properties of various categories of Ru-based luminophores such as $Ru(bpy)_3^{2+}$, Nano materials doped with $Ru(bpy)_3^{2+}$ molecule, and $Ru(bpy)_3^{2+}$ immobilized on Nafion coated electrode are discussed below.

2.1 ECL of $Ru(bpy)_3^{2+}$ complex

The ECL of luminophore/co-reactant system follows only co-reactant mechanism either oxidative-reduction or reductive-oxidation mechanism. The initial discovery of $Ru(bpy)_3^{2+}$ as luminophore shown an avenue in ECL and created a way to study the various ECL reactions. The ECL of $Ru(bpy)_3^{2+}$ along with co-reactant is playing a vital role in broadening the ECL studies. Interestingly the ECL of $Ru(bpy)_3^{2+}$/TPrA system follows both co-reactant and annihilation mechanism. In the case of the co-reactant mechanism TPrA gets oxidized to form $TPA^{\bullet}$ and $Ru(bpy)_3^{2+}$ produces $Ru(bpy)_3^{3+}$ upon oxidation. The $TPA^{\bullet}$ reduces the $Ru(bpy)_3^{3+}$ to excited $Ru(bpy)_3^{2+}$ molecule which emits light (**Figure 5A**). But in the annihilation mechanism, $Ru(bpy)_3^{2+}$ electrochemically oxidizes to $Ru(bpy)_3^{3+}$ and the $TPA^{\bullet}$ directly reacts with $Ru(bpy)_3^{2+}$ to produces $Ru(bpy)_3^{1+}$, then electron transfers from $Ru(bpy)_3^{1+}$ to $Ru(bpy)_3^{3+}$ and generates excited $Ru(bpy)_3^{2+}$ molecule which emits light (**Figure 5B**).

In the reductive-oxidation mechanism, the ECL of $Ru(bpy)_3^{2+}$ occurs along with $S_2O_8^{2-}$, hydrogen peroxide (H_2O_2), and glutathione as co-reactants. The ECL of $Ru(bpy)_3^{2+}$ with glutathione is quite interesting because when the reduced

glutathione (GSH) used as a co-reactant the ECL is ROS dependent. Initially, GSH reacts with reactive oxygen species (ROS) which are produced during the oxygen reduction reaction to forms $GS^{\bullet}$, then $Ru(bpy)_3^{2+}$ reduces to $Ru(bpy)_3^{1+}$ by electrochemically and electron transfers from $Ru(bpy)_3^{1+}$ to $GS^{\bullet}$ leads to the generation of excited $Ru(bpy)_3^{2+}$ which emits light (**Figure 6A**). But in the case

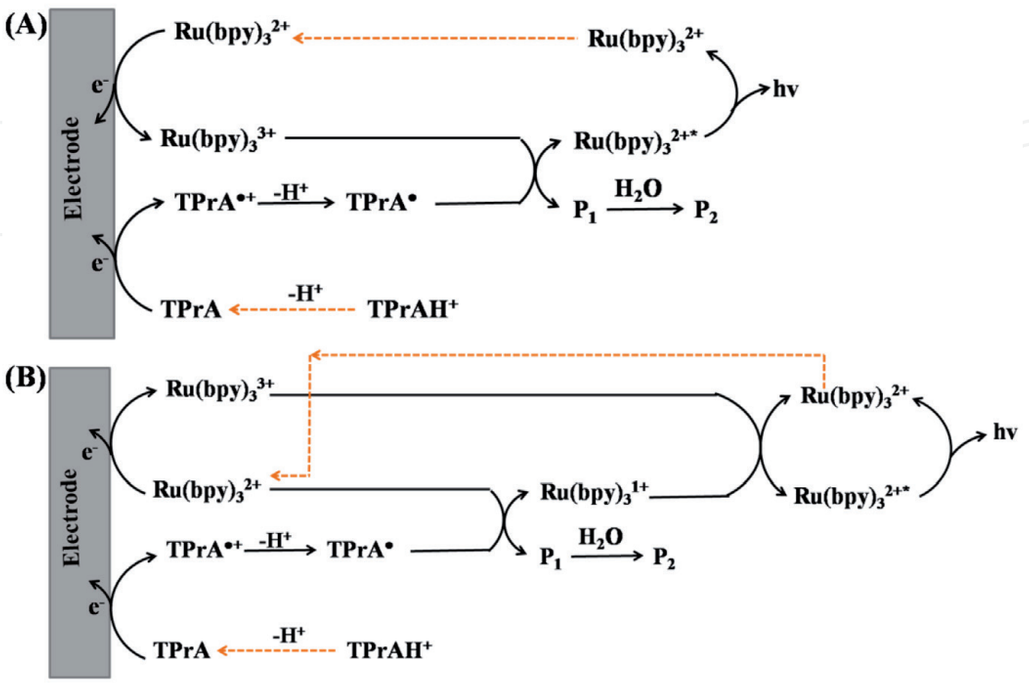

Figure 5.
ECL reaction mechanism of $Ru(bpy)_3^{2+}$/TPrA system (A, B). Copyright © 2002, American Chemical Society.

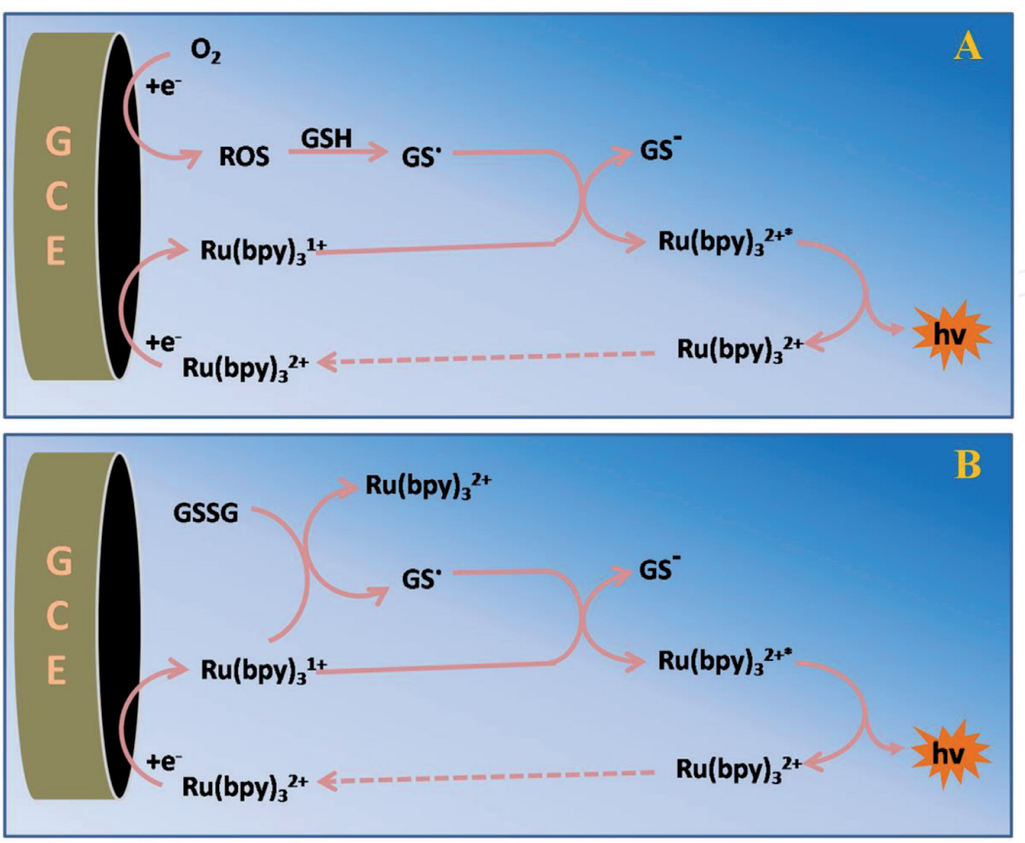

Figure 6.
ECL reaction mechanism of $Ru(bpy)_3^{2+}$/GSH (A) and $Ru(bpy)_3^{2+}$/GSSSG system (B).

of oxidized glutathione (GSSG), ECL is ROS independent, where the $GS^{\bullet}$ forms directly by reacting with $Ru(bpy)_3^{1+}$ after that excited $Ru(bpy)_3^{2+}$ obtained by reacting with $Ru(bpy)_3^{1+}$ and then light emission occurs (**Figure 6B**). However, the *in-situ* generated co-reactants were also utilized in order to study the ECL reaction by taking the $Ru(bpy)_3^{2+}$ as a luminophore [9]. Hence, $Ru(bpy)_3^{2+}$ is acting as a model and benchmark luminophore over the others. However, in order to improve further ECL light emission intensity, researchers were developed $Ru(bpy)_3^{2+}$ derivatives by doping with nano-materials, the results are discussed below.

2.2 ECL of $Ru(bpy)_3^{2+}$-doped with nanomaterials

Doping of nanomaterials with $Ru(bpy)_3^{2+}$ leads to the newer generation of ECL luminophores. The nanoparticles with the functional groups like thiols, amines, and silicates easily covalently bind with $Ru(bpy)_3^{2+}$ and its derivatives to gives highly luminescent luminophores. The $Ru(bpy)_3^{2+}$-doped nanomaterials have several advantages over conventional ECL luminophores. At first, a huge number of luminophore molecules could be encapsulated in a single target molecule site secondly the self-quenching properties of luminophores will be minimized and also the external quenchers like oxygen and water molecules are screened [18]. In this sequence, $Ru(bpy)_3^{2+}$ molecule were covalently linked with doped silica nanoparticles ($Ru(bpy)_3^{2+}$-DSNPs) showed bright ECL signal in 0.1 M acetonitrile with Tetrabutylammonium hexafluorophosphate (MeCN/$TBAPF_6$) potential scan from -1.6 V to +1.5 V [18]. This ECL signal is obtained by annihilation route in the absence of a co-reactant. Further, interesting ECL results were shown by making

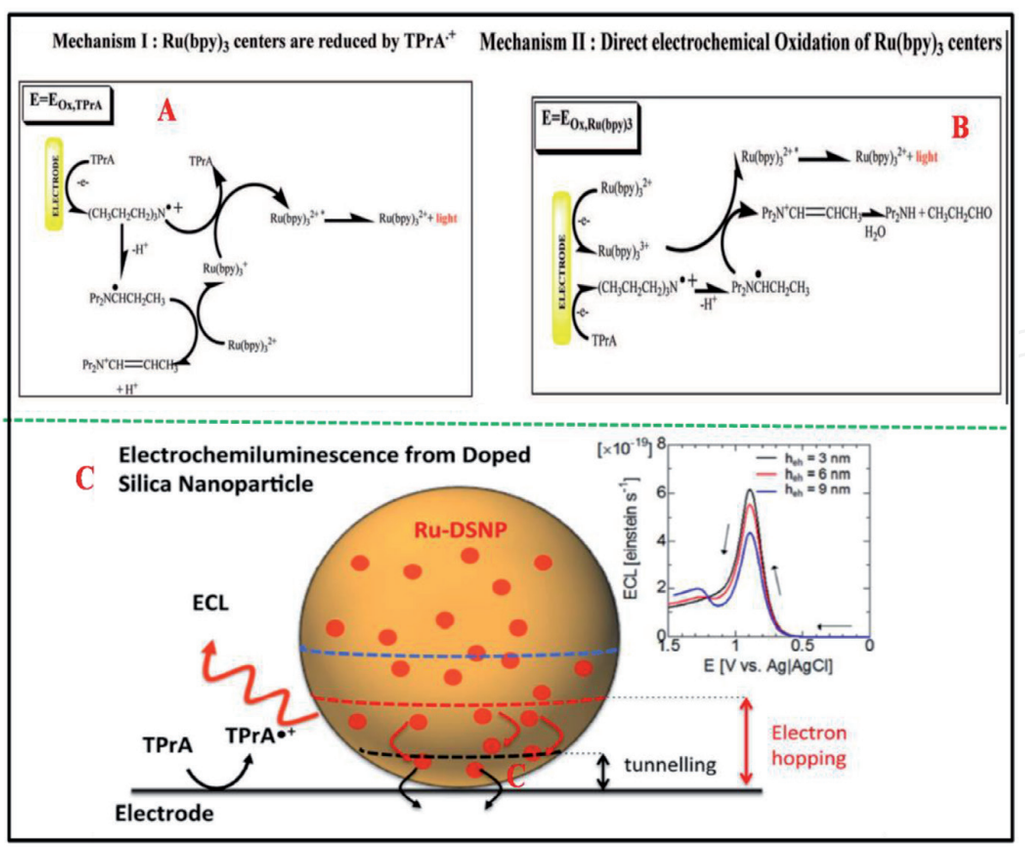

Figure 7.
(A, B) ECL reaction mechanism of DSNP/TPrA system. Copyright © 2009, American Chemical Society. The schematic representation of ECL generation in Ru-DSNP/TPrA system (C) Copyright © 2015, American Chemical Society.

a self-assembled monolayer (DSNPs-SH SAM) on a gold electrode surface in the presence of TPrA [18].

There are two ECL signals were obtained at 0.91 V and 1.23 V during the potential scan from 0 to 1.6 V in 0.1 M phosphate buffer solution (PBS). For the first cycle, the ECL at 0.91 V is much intense than the second peak (at 1.23 V) [18]. In general, the TPrA oxidation on a gold electrode is prevents in PBS because of Au-oxide formation, but DSNPs-SH SAM formation on gold surface creates hydrophobic nature and suppresses the Au-oxide formation. The hydrophobic formation allows the direct oxidation of TPrA and generates more number of $TPA^{\bullet}$ which directly reduces the $Ru(bpy)_3^{2+}$ (in DSNPs) to form $Ru(bpy)_3^{1+}$. Then $Ru(bpy)_3^{1+}$ oxidized by reacting with $TPrA^{\bullet+}$ to generates excited $Ru(bpy)_3^{2+}$ molecule which emits light at 0.91 V (**Figure 7A**). The second peak at 1.23 V is due to electrochemical oxidation of both TPrA and $Ru(bpy)_3^{2+}$ molecule (see **Figure 7B**). On the second cycle onwards the first ECL (at 0.91 V) was disappeared whereas the peak at 1.23 V remains as such because the DSNPs-SH SAM detaches from the electrode surface. This again leads to further Au-oxide growth on Au surface and suppresses the TPrA oxidation, obviously decreases ECL intensity at 0.91 V. Similarly, Ru-DSNP/TPrA exhibits ECL in PBS, the emission is due to the electron hopping between the electrode and $Ru(bpy)_3^{2+}$ as well as between two adjacent $Ru(bpy)_3^{2+}$ presents on DSNP as shown in **Figure 7C**. Another attempt has been carried out by covenant linkage of nitrogen-doped carbon nanodots (NCNDs) with the $Ru(bpy)_3^{2+}$ molecule. The NCNDs acting as co-reactant which is electrochemically oxidized at electrode to produce reactive radicals which have the capability to form excited $Ru(bpy)_3^{2+}$ molecule in generating the ECL [19]. This kind of doping of nanomaterials with the $Ru(bpy)_3^{2+}$offers in enhancing the ECL intensity of $Ru(bpy)_3^{2+}$ and leads to create a new platform for various analytical applications.

2.3 ECL of $Ru(bpy)_3^{2+}$ immobilized on Nafion coated electrodes

The immobilization of $Ru(bpy)_3^{2+}$ into the Nafion coated electrode surface either by chemical or electrochemical methods develops a highly stable and intense solid-state ECL platform. The solid-state ECL has several advantages over solution-phase ECL system. The minute amount of luminophore is sufficient to study the ECL reaction, the luminophore molecule could be regenerated on the electrode surface and also the solubility problem could be overcome. The Nafion is a cation exchange polymer with chemically inert and high thermal stability. It is used for immobilization of positively charged species like $Ru(bpy)_3^{2+}$ molecules. The first reports on immobilization of $Ru(bpy)_3^{2+}$ into the Nafion coated electrodes by Rubinstein and Bard have been extended to lot of studies on ECL reactions [20]. The ECL intensity of $Ru(bpy)_3^{2+}$ controlled by varying the Nafion concentration, thickness of the Nafion coating on the electrode surface and amount of loading of luminophore. For example, the bright ECL observed for $Ru(bpy)_3^{2+}$ immobilized on Nafion Langmuir-Schaefer coated GCE in the presence of TPrA, the highest intense ECL signal obtained for 20 layers coated electrode [21]. In a similar way, $Ru(bpy)_3^{2+}$ immobilized on Nafion coated GCE electrode exhibited three ECL signals in oxygen saturated PBS [22]. The highest ECL intensity was observed when the 6.58 μm thickness of Nafion is coated on the electrode surface. In addition, the modified GCE surfaces also utilized to immobilize the $Ru(bpy)_3^{2+}$ and studied the ECL reactions in order to explore a different kinds of applications [23].

However, the immobilization of $Ru(bpy)_3^{2+}$ on Nafion coated noble metal surfaces like gold electrode shown superior ECL behaviour over the carbon based-electrodes. For instance, $Ru(bpy)_3^{2+}$/Nafion/gold electrode shown unique and

unusual ECL properties [24]. The ECL obtained at very less cathode potentials without co-reactant in the potential region of 0 to 1.2 V in PBS. The ECL spectrum reveals that the observed ECL is because of the formation of excited $Ru(bpy)_3^{2+}$ by electrochemical reaction. Similar way, the ECL of $Ru(bpy)_3^{2+}$ which is presents on Nafion coated nanoporous gold electrode (NPG) is abnormal than the conventional system [25]. In this system $Ru(bpy)_3^{2+}$ molecules is immobilized electrochemically in the acidic electrolyte, as prepared $Ru(bpy)_3^{2+}$/Nafion/NPG composite used to study the ECL in PBS. There is a bright ECL signal obtained at less cathode potential (at 0.1 V *vs* Ag/AgCl) during the potential sweep from 0 to 1.6 V to -1 V. The *in-situ* generated hydroxyl anions (OH^-) from the NPG surface during the reduction playing a key role to gets the ECL at very less potentials. As shown in **Figure 8**, the NPG get oxidized during the potential scan from 0 to 1.6 V to form a gold-hydroxide layer (Au-OH_3). At the same time, the $Ru(bpy)_3^{2+}$ which is immobilized on the electrode surface ($Ru(bpy)_3^{2+}$/Nafion/NPG) also undergoes oxidation to forms $Ru(bpy)_3^{3+}$ intermediate. During the reduction, the Au-OH_3 converts into Au^0 and liberates OH^- ions. The presence of Nafion does not allow diffusion of OH^- ions from electrode surface to bulk electrolyte solution. It results in generation of $OH^\bullet$ by reacting with $Ru(bpy)_3^{3+}$. Then an energetic electron transfer arises from $OH^\bullet$ to $Ru(bpy)_3^{3+}$ leads to the generation of excited $Ru(bpy)_3^{2+}$ molecule which emits light as indicated in **Figure 8**. This type of solid-state ECL which is obtained at very less potentials has several advantages and also has scope in various analytical application points of view. The recent trend in ECL is involving in the development of solid-state ECL for point-of-care applications. The immobilization of luminophores on polymer-coated electrodes helps in creating a solid-state platform in improving the ECL signal intensity as well as could be useful in different analytical applications.

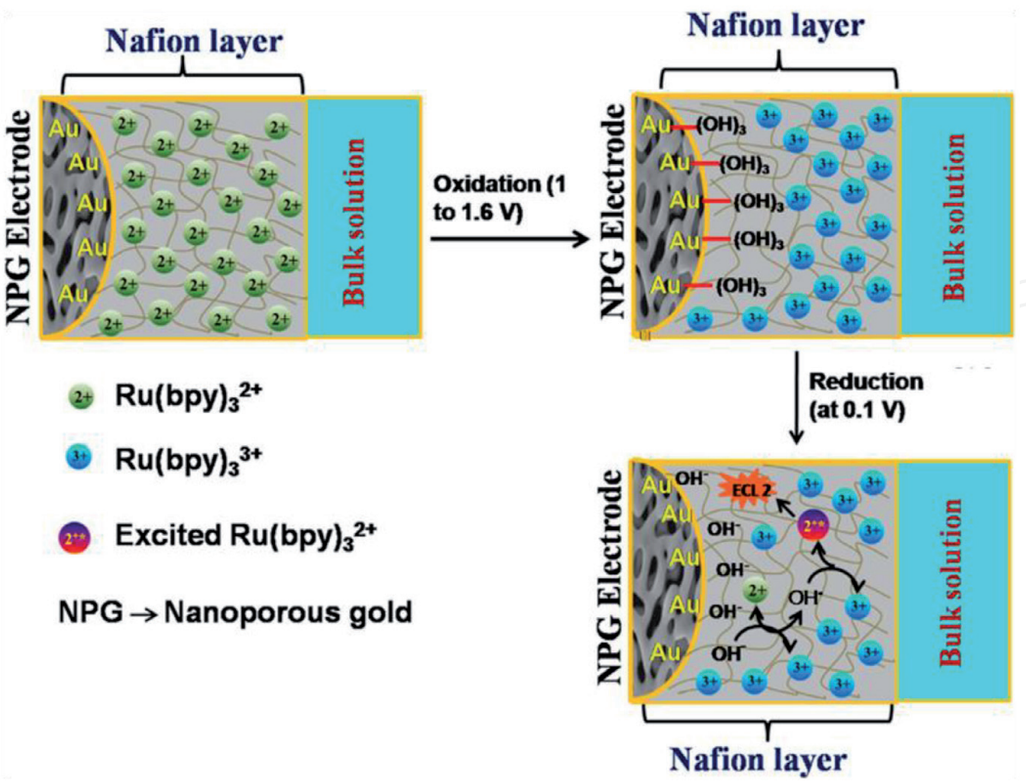

Figure 8.
The schematic representation of solid-sate ECL mechanism involving in $Ru(bpy)_3^{2+}$/Nafion/NPG composite in 0.1 M PBS (pH 7.4).

3. ECL applications

The ECL technique has several distinct advantages over many other detection systems, such as ECL provides excellent sensitivity with a detection limit at very low concentrations (subpicomolar) because of no background signal and an extremely wide dynamic range of orders of magnitude. Also, it allows the coupling of multiple labels to oligonucleotides or peptides without affecting immune reactivity. Finally, the simple instrumentation, the highly sensitive and rapid measurement, high-throughput analysis, made ECL a powerful detecting tool in the ultrasensitive detection of biomarkers. Therefore, it is of more interest among researchers to improve sensitivity and extend the applications of ECL immunoassays, DNA sensing, Bio-imaging, and point-of-care applications. Most of the ECL analysis studies utilize $Ru(bpy)_3^{2+}$ and its derivatives as ECL probe. The role of $Ru(bpy)_3^{2+}$ as an ECL label in different kind of sensing applications are discussed in a detailed way as follows.

3.1 Immunoassays sensing

One of the most important analytical applications of ECL is the use of commercial bioassay based on the $Ru(bpy)_3^{2+}$/TPrA systems. In which the derivatives of $Ru(bpy)_3^{2+}$ are used as ECL-active labels and TPrA as an efficient co-reactant. In the area of clinical diagnosis, multi-component detection is highly adapted than single-component detection. A sandwich-type ECL immunoassay array was used for the detection of multiple protein detection by incorporating the antibody coated single-walled carbon-nanotube (SWCNT) forest micro wells surrounded by the hydrophobic polymer [26]. These carboxylated SWNTs offers a more conductive surface area for the attachment of capture antibodies for Prostate-Specific Antigen (PSA) and interleukin-6 (IL-6) at the bottom of different micro-wells by amidization. The ECL signals were measured with the CCD camera, and the limit of detection for the PSA and IL-6 was observed to be 1 pg mL^{-1} and 0.25 pg mL^{-1}, which is better than the commercially available bead-based protein measurement systems. As shown in **Figure 9**, the ECL enzyme-linked immunosorbent assay was developed and used to determine the methyl-cytosine in DNA [27]. The anti-methyl cytosine antibody conjugated with acetylcholinosterase, in which the acetylcholinosterase converted acetylthiocholine (substrate) to thiocholine (product). This thiocholine

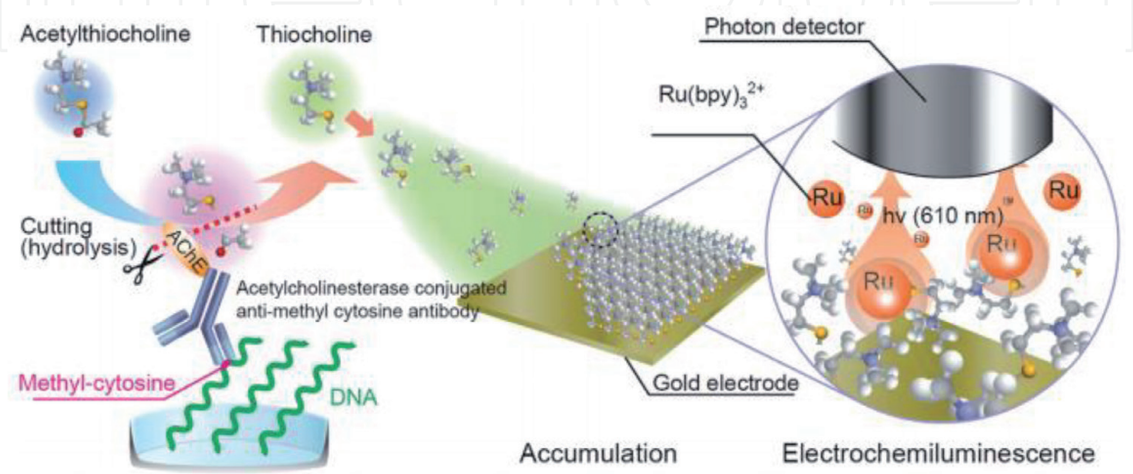

Figure 9.
Scheme for the principle of ECL determination of methyl-cytosine. Copyright © 2012, American Chemical Society.

is a bifunctional molecule that exhibits both the effect of ECL acceleration and surface accumulation *via* gold-thiol binding. Due to the accumulation and acceleration effect on ECL, the quantitative measurement of methyl-cytosine was found to have higher sensitivity with the linear range from 1 pmol to 100 pmol, which is sufficient to achieve the real DNA measurements. In order to increase the ECL emission intensity of the $Ru(bpy)_3^{2+}$-derivatives based ECL system, a novel and the self-enhanced ECL luminophore of the Ru(II) complex was developed by using the poly(ethylenimine) as a co-reactant, and also to form a coil-like nanocomposite with [$Ru(bpy)_2$(5-NH_2-1,10-phen)$^{2+}$] [28]. By adopting the self-enhanced ECL luminophore in the sandwich-type ECL immunoassay, ultrasensitive detection of apurinic/apyrimidinic endonuclease-1 was demonstrated for the first time with the improved detection sensitivity from pg mL^{-1} to fg mL^{-1}.

The ultrasensitive detection of human C-reactive protein (CPR) was demonstrated by the addition of multiple $Ru(bpy)_3^{2+}$ to a single antibody with the encapsulation of a hydrophobic compound in the polystyrene microbeads [29]. With the sandwich-type immunoassay, a high sensitive CPR detection has been achieved with the detection limit as low as 0.01 μg mL^{-1}. The obtained LOD was found to be lower than the presently available high-sensitive CPR assay systems. Based on the previous work, a similar idea of holding multiple labels was developed by the preparation of sub-micrometer-sized liposomes containing $Ru(bpy)_3^{2+}$ as the ECL-active labels for CPR immunoassay. The addition of 0.1 M TPrA in the electrolytic solution (0.1 M PBS) containing 0.1 M NaCl and 1% Triton X-100, the release of the ECL label $Ru(bpy)_3^{2+}$ from the liposome can be realized. The above described approach allows the bioassay to be carried out in the aqueous solutions, which is compatible with the currently available commercial ECL instrumentation. Later, this work has been extended to the application in detecting hemagglutinin, which plays a significant role in the influenza virus infection.

3.2 DNA sensors

DNA detection is of great importance in the areas of clinical testing, forensics analyses, gene expression analysis, and biological warfare agent detections. ECL has been used as a powerful analytical tool in the DNA probe assays. Similar to the ECL immunoassays, DNA probe assays is also based on the $Ru(bpy)_3^{2+}$/TPrA ECL Systems. Generally, ECL DNA probe assays can be classified into two types: (1) Label-free, and (2) Label ECL detection [13, 30]. In the year of 1991, Blackburn *et al.* first reported the use of ECL of $Ru(bpy)_3^{2+}$ in developing the immunoassay and DNA probe assay for clinical diagnostics [31]. The general principle for the detection of DNA using the ECL label is outlined in **Figure 10A**. In this strategy, the ssDNA is immobilized on the electrode surface, and followed by attachment of the complementary target strand ssDNA tagged with the ECL label hybridizes with the immobilized ssDNA. Then the electrode assembly is placed in an electrolyte solution containing the co-reactant and allowed for the measurement of ECL [32]. Later, Xu *et al.* developed the ECL biosensor for the detection of DNA, based on the adsorption reaction on the film modified electrode surface. In this work, firstly tagged ssDNA was immobilized on the electrode surface coated with an organized aluminium phosphate film. By immersing the film in the DNA solution, the amount of immobilized DNA-$Ru(bpy)_3^{2+}$ was determined by the ECL emission resulting from the electrochemical oxidation of $Ru(bpy)_3^{2+}$ and TPrA in the solution [33]. An ultrasensitive ECL method has been developed for the detection of DNA hybridization by using polystyrene microspheres/beads, as the carriers of a huge numbers of hydrophobic ECL-active labels $Ru(bpy)_3[B(C_6F_5)_4]$ as shown in **Figure 10B**. The label-free ECL DNA detection was achieved based on the catalytic oxidation of

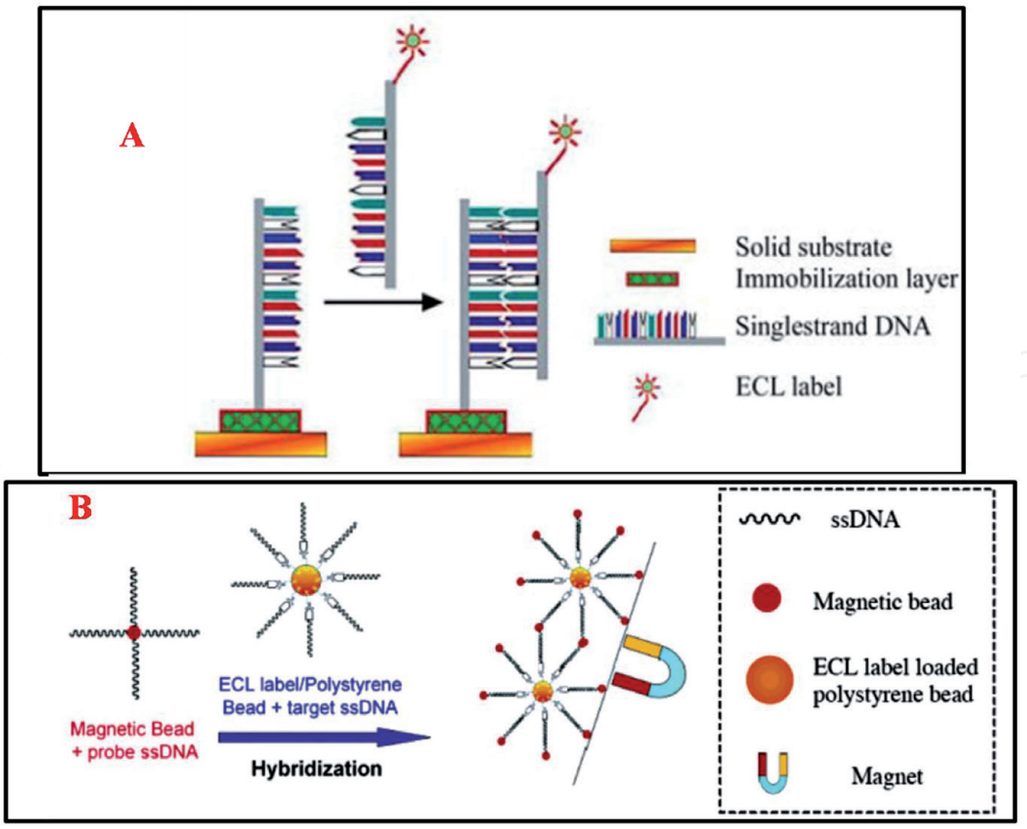

Figure 10.
Schematic diagram of solid-state ECL detection of DNA hybridization (A) Copyright © 2003, American Chemical Society. Schematic diagram of DNA hybridization on a polystyrene bead as the ECL label carrier and a magnetic bead for the separation of analyte-contained ECL label/polystyrene beads (B) Copyright © 2004, American Chemical Society.

guanine and adenine base using a glassy carbon electrode modified CNT/Nafion/ $Ru(bpy)_3^{2+}$ has been reported [34]. The ECL signals for the dsDNA and their denatured counterparts could be detected with a very lower concentration of 30.4 nM and a single-base mismatch gene p53 gene sequence segment was detected with the concentration of 0.4 nM.

3.3 Bio-imaging

An important breakthrough in the analytical application of the ECL technique is the combination of this transduction technique with microscopy. The combination of ECL along with a microscope leads to marvelous investigation in imaging of cell and tissue of living beings, nanomaterial imaging, and imaging of single-particle collisions [35]. The ECL imaging setup consists of (i) bright-field microscopy, (ii) a potentiostat, and (iii) the charge-coupled device (CCD) for recording the image [36]. The ECL emission signal is directly proportional to the concentration of the luminophore and the co-reactant; hence, the ECL technique enables the sensitive detection and quantification of both luminophore and the co-reactant. This widens the application of the ECL technique in detecting various biomolecules, such as proteins, enzymatic substrates, and nucleic acids.

In the most of Bio-imaging analysis, the $Ru(bpy)_3^{2+}$ molecule utilized as the ECL luminophore due to high quantum efficiency. For example, Valenti *et al.* used $Ru(bpy)_3^{2+}$ as ECL label in imaging the single Chinese Hamster Ovary (CHO) cell [37]. Initially, the cells are cultured on a glassy carbon electrode and incubated with biotin X which is capable of reacting with amino groups of the protein. Then the

$Ru(bpy)_3^{2+}$ molecule is labeled with streptavidin (SA@Ru). The biotin group reacts with SA@Ru and attaches the cell membrane throughout the ECL measurements as shown in **Figure 11a**. Further similar strategy used to imaging the MCF10A cell, in this case, CNT electrode used to incubate the cells and $Ru(bpy)_3^{2+}$ labeled with monolocal antibody (Ab@Ru) serves as ECL probe as shown in **Figure 11b**. The ECL imaging has performed in the PBS (pH 7.4) containing TPrA at anodic applied potential of 1.35 V. **Figure 11c** and **d** displays the PL image and ECL image of CHO cells on GC electrode. PL image shows a spatial distribution of SA@Ru labels with an entire cell appearance (**Figure 11c**). But in the case of the ECL image, the cell border glows with red color whereas cell nucleus is dark (**Figure 11d**). This is because ECL labels (SA@Ru) specifically located on the target cell. Thus the ECL is more specific to image single cell over the PL. Similar kind of results was observed in the case of MCF10A cell, the PL shows the emission of the light entire the cell (**Figure 11e**) whereas ECL image emits light only at a specified cell membrane (**Figure 11f**). Apart from this, the microelectrode arrays also used in single cell imaging by adopting ECL strategy [38].

3.4 Aptamer-based sensors

Aptamers are peptide or oligonucleotide molecules and also a functional DNA or RNA structures. Aptamers can specifically bind with small molecules as well as peptides and proteins. Aptamers exhibit remarkable advantages over conventional molecular recognition systems like antigen-antibody interactions because of their easier synthesis protocols, simple labeling, and high stability [30]. The combination ECL tool along with aptamer showed attractive results in biosensor applications and several aptamer-based ECL biosensors have been developing to detect small

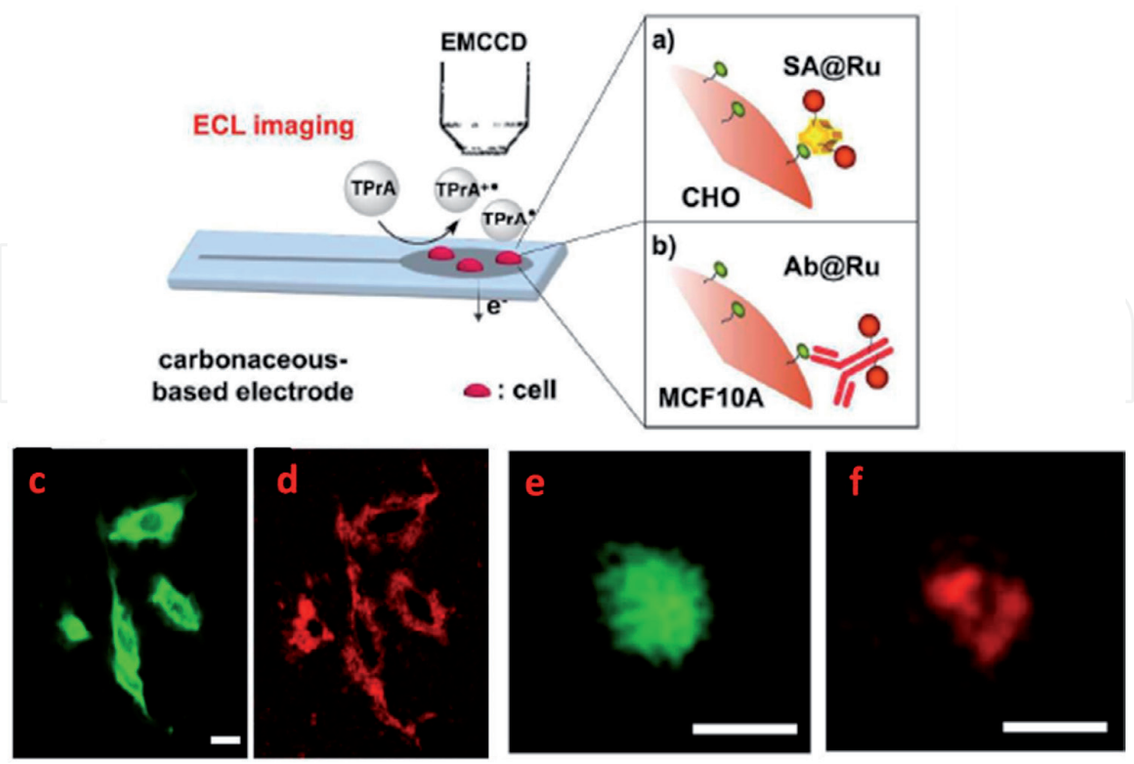

Figure 11.
The schematic illustration of principle involving in ECL imaging (a, b) PL and ECL images of SA@Ru attached CHO cells (c, d) and PL, ECL images of Ab@Ru attached MCF10A cells (e, f) Copyright © 2017, American Chemical Society.

molecules. In most of the aptamer-based ECL sensors, the $Ru(bpy)_3^{2+}$ molecule and its derivatives using as ECL label along with TPrA as co-reactant, due to unique and high quantum efficiency. For example, thrombin was detected by $Ru(bpy)_3^{2+}$ molecule as ECL probe, in this the captured aptamers with AuNPs labels were immobilized on thiolated ITO electrode via Au-S linkage and then catches the target aptamer [39]. After that, the $Ru(bpy)_3^{2+}$ molecule label was tagged with aptamer and studied the ECL experiments in TPrA containing PBS. This ECL strategy allows the detection of thrombin at a very low level with 10 nM of LOD. In addition to this, thrombin is detected by using tris(1, 10-phenanthroline) ruthenium ion ($Ru(phen)_3^{2+}$) intercalated into double standard DNA (dsDNA) as ECL sensing probe along with antithrombinthiolated aptamer [40].

The modification of gold electrode for ECL sensor is shown in **Figure 12**. Initially, the gold electrode soaked in a solution containing 2-mercaptoethanol in order to block the electrode exposing surface, then antithrombin aptamer adsorbed on the electrode surface as shown in **Figure 12A**. After that ds-DNA is placed between the aptamer and ss-DNA to intercalate the luminophore molecule (**Figure 12B**). To intercalate the $Ru(phen)_3^{2+}$ into the ds-DNA, the modified electrode is dipped in the ds-DNA solution. ECL experiments performed in PBS (pH 7.5) containing TPrA, a sharp ECL signal observed at 1.1 V which is due to the energetic electron transfer between reactive intermediates of $Ru(phen)_3^{2+}$ and TPrA. The ECL intensity decreases during the addition of 5 pM of thrombin into the electrolytic solution [40]. The decrease in the ECL signal is due to the detachment of $Ru(phen)_3^{2+}$ molecule from ds-DNA and occupation by thrombin as indicated in **Figure 12D**. This ECL methodology provides to sense the thrombin in the range of 0.05 to 50 pM with the detection limit of 0.02 pM.

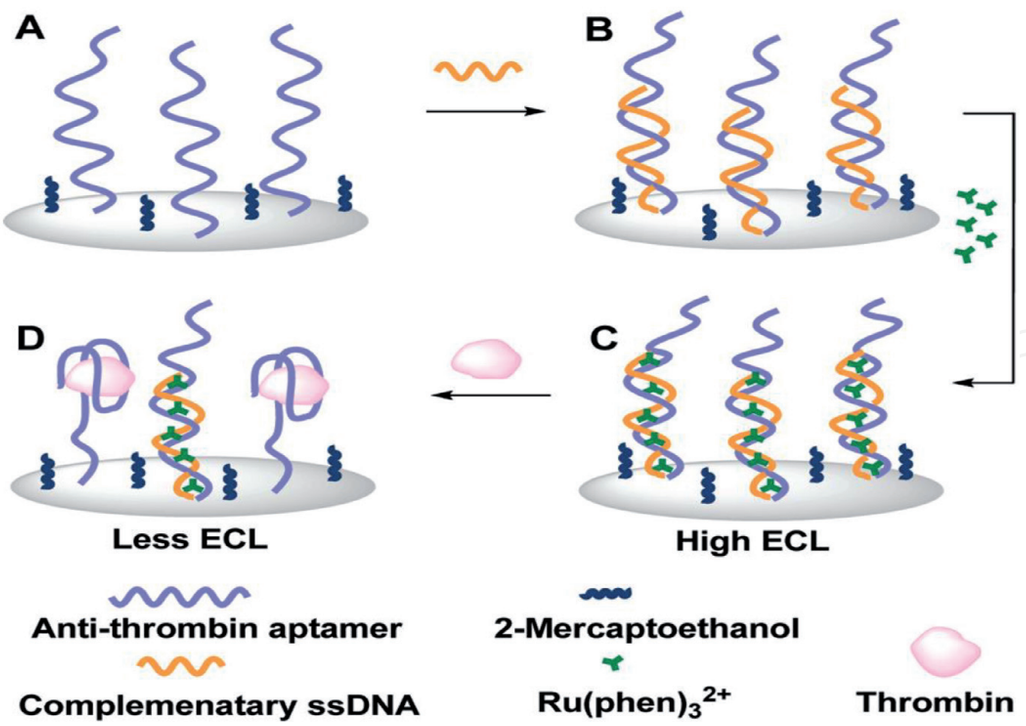

Figure 12.
Schematic representation of the principle involving in thrombin detection by aptamer-based ECL sensor. (A) The adsorption of thiolatedantithrombin aptamer on and the 2-mercaptoethanol block to the electrode. (B) The formation of the ds-DNA between aptamer and its complementary ss-DNA. (C) The intercalation of $Ru(phen)_3^{2+}$ into the ds-DNA sequence. (D) Dissociation of ds-DNA and release of $Ru(phen)_3^{2+}$ due to the interaction between thrombin and its aptamer, resulting in the decreased ECL emission which was used to quantify thrombin. Copyright © 2009, American Chemical Society.

3.5 Metal ion detection

Heavy metal ions such as Hg, Pb, Sn and, Cd are highly toxic and undegradable, causes serious health issues to humans [41]. The heavy metal ions migrate into the soil from industrial wastage, pesticides, fertilizers, spilling of petrochemicals. As migrated metal ions contaminants food and food security has become a worldwide worry. For the past few decades, the ECL has been using as one of the leading analytical technique in detecting such metal pollutants in food as well as soil. In this context, bipolar ECL (BP-ECL) has employed in detecting the Cu^{2+} and Cd^{2+} ions [42]. This method offers high sensitivity, low cost and provides both qualitative and quantitative information. The mixed luminophores of $Ru(bpy)_3^{2+}$ molecule along with $Ir(ppy_3)$ acting as ECL sensing probe. The multicoloured ECL emission is obtained at 540 nm (green) 610 nm (orange) at the driving potential of 5.5 V in the presence of both luminophores along with TPrA. The BP-ECL method offers the determination of Cd^{2+} ion in the range of 1 μM to 75 μM with the limit of detection of 0.094 μM (10 ppm) and also the Cu^{2+} ion detected within the dynamic range of 0.1 μM to 1/75 μM with 0.008 μM of LOD. In addition to this, $Ru(bpy)_3^{2+}$ linked with crown ethers used as an ECL probe to determine the Pb (II) ion [43], and also the solid-state ECL has been employed by using $Ru(bpy)_3^{2+}$ as an ECL probe to detect the Pb (II) ion in trace level [44]. The Pb(II) ion detected in the linear range of $1x10^{-6}$ μM to $1x10^{2}$ μM by ECL turn-off method. Another class of heavy metal Hg (II) ion detected by the employing cathodic ECL of $Ru(bpy)_3^{2+}$/NHS system [45]. This system includes the zero background signal, wide dynamic detection range and highly sensitive and selective to detect Hg (II) in physiological pH. The ECL intensity gradually increases upon each addition of Hg (II) ion into the solution in the linear range of 0.001 μM to 20 μM. This cathodic ECL method shows the limit of detection of 0.1 nM towards Hg (II) detection without interfering with other metal ions.

3.6 Point-of-care applications

Now a day's ECL becomes one of the dynamic, highly sensitive, and well established tools in point-of-care applications. ECL strategy has been used in the fabrication of point-of-care testing devices (POCT) like ECL detectors,

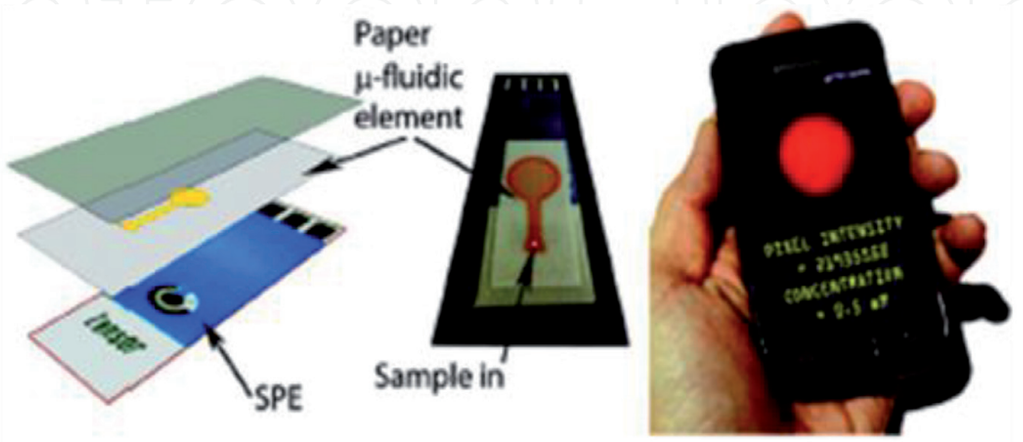

Figure 13.
Schematic diagram of a paper-based microfluidic ECL sensor. Using inkjet-printed paper fluidic substrates and screen-printed electrodes, this ECL sensor can be read with mobile phone cameras. Copyright © 2011, American Chemical Society.

bipolar ECL devices, wireless ECL, and microfluidic chips. These POCT devices are designed by using luminophores as sensing probes, the $Ru(bpy)_3^{2+}$ is a benchmark material among the various luminophores due to high quantum efficiency. The portable devices with lower prices could be useful for hospital/nonhospital purposes. The paper-based ECL sensors are one of the best cheapest ECL devices to detect biological molecules at trace level [46]. This type of ECL sensor has advantages that possess high sensitivity, selectivity, and rapid detection. The paper-based microfluidic devices made by patterning the papers into hydrophilic channels separated by hydrophobic barriers which allow the uniform distribution of samples into the regions. The photoresist materials like wax, polydimethylsiloxane, alkylketene dimer, polystyrene, poly(o-nitrobenzyl methacrylate), fluorochemicals, methylsilsesquioxane, and toner used as hydrophobic barriers [47]. Yan *et al.* developed a paper-based ECL 3D device. The device made with two different kinds of patterned cellulose papers called paper-A and paper-B. These two papers were converted into screen printed working paper electrode (SPWPE) by using carbon paste, the Ag/AgCl used as a reference electrode. As fabricated SPWPE used for point-of-care applications. The $Ru(bpy)_3^{2+}$ used as an ECL sensing probe along with TPrA as a co-reactant. The ECL signal intensity enhanced by 10-fold in the presence of CEA antibody [46]. Apart from this, microfluidic-based ECL sensor shown much attention in point-of-care applications due to flexibility, cheaper cost, portability, and porosity. **Figure 13** represents the schematic illustration of paper-based the microfluidic ECL sensor device. The inkjet printing is used to prepare microfluidic substrate, then it is combined with screen-printed electrodes which produce portable, disposable and photo-detectorless ECL sensor device. Initially, the printed paper based microfluidic filled with 13 μl of 10 mM $Ru(bpy)_3^{2+}$ solution and allowed to dry. After drying the paper microfluidic substrate is further laminated on Zensor screen-printed electrode (SPE) with 80 μm thickness. The small slit is made during the lamination to introduce the sample. The $Ru(bpy)_3^{2+}$ serves as ECL sensor probe and the mobile phone used to detect the ECL signal. The conventional photodetector used to detect the DBAE and NAD in the range of 0.9 μM and 72 μM respectively. In addition to this, the wireless ECL and bipolar ECL sensors also used as in fabricating the point-of- care testing devices for hospital/nonhospital purposes.

4. Conclusions and future perspective

The $Ru(bpy)_3^{2+}$ and its derivatives play a crucial role in the development of ECL based sensor devices. Due to its chemical stability, confined electrochemical behaviour, and high ECL quantum efficiency, $Ru(bpy)_3^{2+}$ complex acts as one of the best model luminophores. The variety of ECL experiments were studied by using different forms of $Ru(bpy)_3^{2+}$ like Ru-chelates, doped nanoparticles-$Ru(bpy)_3^{2+}$ and polymer-$Ru(bpy)_3^{2+}$ complex and used as an ECL label in detecting the immunoassays, DNA detection, aptamer sensing, bio-imaging, and metal ion sensing. The dynamic and progressive ECL of $Ru(bpy)_3^{2+}$ also offers to use it as a sensing probe in point-of-care testing devices such as portable ECL sensor devices, wireless ECL kits, paper-based microfluidic ECL devices, and bipolar ECL devices. Moreover, these kinds of ECL-based sensor kit will extent to the development of sensing of food adulterants to detect the toxic, hazardous and harmful compounds in food. In near future, the fabrication of $Ru(bpy)_3^{2+}$ based ECL sensor kits will be useful in various clinical diagnostic applications, pharmaceutical, and other point-of-care applications.

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Conflict of interest

The authors declare there are no conflicts of interest.

Author details

Chikkili Venkateswara Raju[1,2], Mathavan Sornambigai[1,2] and Shanmugam Senthil Kumar[1,2]*

1 Electrodics and Electrocatalysis Division, CSIR-Central Electrochemical Research Institute (CECRI), Karaikudi, Tamilnadu, India

2 Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India

*Address all correspondence to: ssenthilmugam@gmail.com; ssenthilkumar@cecri.res.in

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References

[1] M.M. Richter: Electro chemiluminescence (ECL).Chem.Rev. 2004;104:3003-3036. DOI:10.1021/cr020373d

[2] D.M. Hercules: Chemiluminescence Resulting from Electrochemically Generated Species. Science. 1964;145:808-809. DOI:10.1126/science.145.3634.808

[3] H. Tachikawa, A.J. Bard: Electrogenerated chemluminescence. Effect of solvent and magnetic field on ECL of rubrene systems. Chem. Phys. Lett. 1974;26:246-251. DOI:10.1016/0009-2614(74)85407-2

[4] N.E. Tokel, A.J. Bard: Electrogenerated chemiluminescence. IX. Electrochemistry and emission from systems containing tris(2,2'-bipyridine) ruthenium(II) dichloride. J. Am. Chem. Soc. 1972;94:2862-2863. DOI:10.1021/ja00763a056

[5] W. Miao, J.-P. Choi, A.J. Bard: Electrogenerated Chemiluminescence 69: The Tris(2,2'-bipyridine) ruthenium(II), ($Ru(bpy)_3^{2+}$)/ Tri- n -propylamine (TPrA) System. A New Route Involving $TPrA^{\bullet+}$ Cation Radicals.J. Am. Chem. Soc. 2002;124:14478-14485. DOI:10.1021/ja027532v

[6] H.S. White, A.J. Bard: Electrogenerated chemiluminescence. 41. Electrogenerated chemilum inescence and chemiluminescence of the $Ru(2,2^1\text{-bpy})_3^{2+}$-$S_2O_8^{2-}$ system in acetonitrile-water solutions.J. Am. Chem. Soc. 1982;104:6891-6895. DOI:10.1021/ja00389a001

[7] I. Rubinstein, A.J. Bard: Electrogenerated Chemiluminescence. 37. Aqueous Ecl Systems Based on Ru(2,2'-bipyridine)$_3^{2+}$ and Oxalate or Organic Acids Israel. J. Am. Chem. Soc. 1981;103:512-516.DOI.10.1021/ja00393a006

[8] Y. Yuan, S. Han, L. Hu, S. Parveen, G. Xu: Coreactants of tris(2,2′-bipyridyl) ruthenium(II) Electrogenerated Chemiluminescence.Electrochim. Acta. 2012;82:484-492. DOI:10.1016/j.electacta.2012.03.156

[9] Irkham, A. Fiorani, G. Valenti, N. Kamoshida, F. Paolucci, Y. Einaga: Electrogenerated Chemiluminescence by in Situ Production of Coreactant Hydrogen Peroxide in Carbonate Aqueous Solution at a Boron-Doped Diamond Electrode. J. Am. Chem. Soc. 2020;142:1518-1525. DOI:10.1021/jacs.9b11842

[10] P. McCord, A.J. Bard: Electrogeneratedchemiluminescence. Part 54.Electrogenerated chemiluminescence of ruthenium(II) 4,4'-diphenyl-2,2'-bipyridine and ruthenium(II) 4,7-diphenyl-1,10-phenanthroline systems in aqueous and acetonitrile solutions. J. Electroanal. Chem. 1991;318:91-99. DOI:10.1016/0022-0728(91)85296-2

[11] P. Li, Z. Jin, M. Zhao, Y. Xu, Y. Guo, D. Xiao: Self-enhanced electrogenerated chemiluminescence of ruthenium(II) complexes conjugated with Schiff bases. Dalton Trans. 2015;44:2208-2216. DOI:10.1039/C4DT03310H

[12] S.E.K. Kirschbaum, A.J. Baeumner: A review of electrochemiluminescence (ECL) in and for microfluidic analytical devices. Anal Bioanal Chem. 2015;407:3911-3926. DOI:10.1007/s00216-015-8557-x

[13] Z. Liu, W. Qi, G. Xu: Recent advances in electrochemiluminescence. Chem. Soc. Rev. 2015;44:3117-3142. DOI:10.1039/c5cs00086f

[14] H. Cui, X.-Y. Zhao, X.-Q. Lin: Cathodic electrochemiluminescence of $Ru(bpy)_3^{2+}$/Nafion coated on graphite oxide electrode in purely aqueous

solution. Luminescence. 2003;18:199-202. DOI:10.1002/bio.724

[15] X. Chen, Y. Liu, Q. Ma: Recent advances in quantum dot-based electrochemiluminescence sensors.J. Mater. Chem. C. 2018;6:942-959. DOI:10.1039/C7TC05474B

[16] H. Wei, E. Wang: Electro chemiluminescence of tris(2,2′-bipyridyl)ruthenium and its applications in bioanalysis: A review. Luminescence. 2011;26:77-85. DOI:10.1002/bio.1279

[17] Y. Chen, J. Xu, J. Su, Y. Xiang, R. Yuan, Y. Chai: In Situ Hybridization Chain Reaction Ampli fi cation for Universal and Highly Sensitive Electrochemiluminescent Detection of DNA. *Anal. Chem.* 2012;84:7750-7755. DOI:10.1021/ac3012285

[18] S. Zanarini, E. Rampazzo, L. Della Ciana, M. Marcaccio, E. Marzocchi, M. Montalti, F. Paolucci, L. Prodi: $Ru(bpy)_3^{2+}$ Covalently Doped Silica Nanoparticles as Multicenter Tunable Structures for Electrochemiluminescence Amplification. J. Am. Chem. Soc. 2009;131:2260-2267. DOI:10.1021/ja8077158

[19] S. Carrara, F. Arcudi, M. Prato, L. De Cola: Amine-Rich Nitrogen-Doped Carbon Nanodots as a Platform for Self-Enhancing Electrochemiluminescence. Angew. Chem. Int. Ed. 2017;56:4757-4761. DOI:10.1002/anie.201611879

[20] I. Rubinstein, A.J. Bard: Polymer films on electrodes. 5. Electrochemistry and chemiluminescence at Nafion-coated electrodes. J. Am. Chem. Soc. 1981; 103:5007-5013. DOI:10.1021/ja00407a006

[21] P. Bertoncello, L. Dennany, R.J. Forster, P.R. Unwin: Nafion–Tris(2-2'-bipyridyl)ruthenium(II) Ultrathin Langmuir–Schaefer Films: Redox Catalysis and Electrochemiluminescent Properties.*Anal. Chem*. 2007;79:7549-7553. DOI:10.1021/ac070811m

[22] L. Zheng, Y. Chi, Q. Shu, Y. Dong, L. Zhang, G. Chen: Electrochemiluminescent Reaction between $Ru(bpy)_3^{2+}$ and Oxygen in Nafion Film.J. Phys. Chem. C. 2009;113:20316-20321. DOI:10.1021/jp902239j

[23] Z. Guo, S. Dong: Electrogenerated chemiluminescence determination of dopamine and epinephrine in the presence of ascorbic acid at carbon nanotube/nafion-$Ru(bpy)_3^{2+}$ composite film modified glassy carbon electrode. Electroanalysis. 2005;17:607-612. DOI:10.1002/elan.200403129

[24] B. Qi, X.-B. Yin, Y. Du, X. Yang: Unique electrochemiluminescence behavior of $Ru(bpy)_3^{2+}$ in a gold/Nafion/$Ru(bpy)_3^{2+}$ composite.Mater. Lett. 2008;62:458-461. DOI:10.1016/j.matlet.2007.05.065

[25] C. Venkateswara, M. Sornambigai, S. Senthil: Unraveling the reaction mechanism of co-reactant free in-situ cathodic solid state ECL of Ru $(bpy)_3^{2+}$ molecule immobilized on Nafion coated nanoporous gold electrode.Electrochim. Acta. 2020;358:136920. DOI:10.1016/j.electacta.2020.136920

[26] N.P. Sardesai, J.C. Barron, J.F. Rusling: Carbon nanotube microwell array for sensitive electrochemiluminescent detection of cancer biomarker proteins.*Anal. Chem*. 2011;83:6698-6703. DOI:10.1021/ac201292q

[27] R. Kurita, K. Arai, K. Nakamoto, D. Kato, O. Niwa: Determination of DNA methylation using electrochemiluminescence with surface accumulable coreactant.*Anal. Chem*. 2012;84:1799-1803. DOI:10.1021/ac202692f

[28] M. Zhao, X.D.Y.Q. Chai, J. Han, G.F. Gui, R. Yuan, Y. Zhuo: A reagentless electrochemiluminescent immunosensor for apurinic/apyrimidinic endonuclease 1 detection based on the new $Ru(bpy)_3^{2+}$/bi-arginine system.Anal. Chim. Acta. 2014;846:36-43. DOI:10.1016/j.aca.2014.07.017

[29] W. Miao, A.J. Bard:Electrogenerated Chemiluminescence. C-Reactive Protein Determination at High Amplification with $[Ru(bpy)_3]^{2+}$-Containing Microspheres. Anal. Chem.2004;76:7109-7113. DOI:10.1021/ac048782s

[30] L. Hu, G. Xu: Applications and trends in electrochemiluminescence. Chem. Soc. Rev. 2010;39:3275-3304. DOI:10.1039/b923679c

[31] F. Zhang, H. Chen, P.G. He, Y.Z. Fang: Research on DNA electrochemiluminescence biosensing. Chinese Journal of Analytical Chemistry. 2013;41:1-9. DOI:10.1016/S1872-2040(13)60618-0

[32] R. Pyati, M.M. Richter: ECL—Electrochemical luminescence, Annu. Rep. Prog. Chem., Sect. C: Phys. Chem. 2007;103:12-78. DOI:10.1039/B605635K

[33] W. Miao, A.J. Bard: DNA Hybridization Detection at High Amplification with $[Ru(bpy)_3]^{2+}$-Containing Microspheres. Anal. Chem.2004;76:5379-5386. DOI:10.1021/ac0495236

[34] X.H. Xu, A.J. Bard: Immobilization and Hybridization of DNA on an Aluminum(III) Alkanebisphosphonate Thin Film with Electrogenerated Chemiluminescent Detection.J. Am. Chem. Soc. 1995;117:2627-2631. DOI:10.1021/ja00114a027

[35] A. Zanut, A. Fiorani, S. Rebeccani, S. Kesarkar, G. Valenti:Electrochemil uminescence as emerging microscopy techniques. Anal Bioanal Chem. 2019;411:4375-4382. DOI:10.1007/s00216-019-01761-x

[36] J. Zhang, S. Arbault, N. Sojic, D. Jiang: Electrochemiluminescence Imaging for Bioanalysis.Annu Rev Anal Chem.2019;12:275-295. DOI:10.1146/annurev-anchem-061318-115226

[37] G. Valenti, S. Scarabino, B. Goudeau, A. Lesch, M. Jovic, M. Sentic, S. Rapino, S. Arbault, F. Paolucci, N. Sojic: Single Cell Electrochemiluminescence Imaging:From the Proof-of-Concept to Disposable Device-Based Analysis.J. Am. Chem. Soc. 2017;139:16830-16837 (2017). DOI:10.1021/jacs.7b09260

[38] M. Sentic, F. Virgilio, A. Zanut, D. Manojlovic, S. Arbault, M. Tormen, N. Sojic, P. Ugo: Microscopic imaging and tuning of electrogenerated chemiluminescence with boron-doped diamond nanoelectrode arrays.Anal Bioanal Chem.2016;408:7085-7094. DOI:10.1007/s00216-016-9504-1

[39] L. Fang, Z. Lü, H. Wei, E. Wang: A electrochemiluminescence aptasensor for detection of thrombin incorporating the capture aptamer labeled with gold nanoparticles immobilized onto the thio-silanized ITO electrode. Anal. Chim. Acta. 2008;628:80-86. DOI:10.1016/j.aca.2008.08.041

[40] X. Yin, Y. Xin, Y. Zhao: Label-Free Electrochemiluminescent Aptasensor with Attomolar Mass Detection Limits Based on a $Ru(phen)_3^{2+}$-Double-Strand DNA Composite Film Electrode. Anal. Chem. 2009;81:9299-9305. DOI:10.1021/ac901609g

[41] R.A. Wuana, F.E. Okieimen: Heavy Metals in Contaminated Soils: A Review of Sources , Chemistry , Risks and Best Available Strategies for Remediation. International Scholarly Research Network. 2011; DOI:10.5402/2011/402647

[42] S. Carrara, C.F. Hogan: Multi-colour bipolar electrochemiluminescence for heavy metal ion detection. Chem. Commun. 2019;55:1024-1027. DOI:10.1039/c8cc08472f

[43] B.D. Muegge, M.M. Richter: Electrochemiluminescent Detection of Metal Cations Using a Ruthenium (II) Bipyridyl Complex Containing a Crown Ether Moiety. Anal. Chem. 2002;74:547-550.DOI:10.1021/ac010872z

[44] X. Shan, T. Pan, Y. Pan, W. Wang, X. Chen, X. Shan: Highly Sensitive and Selective Detection of Pb(II) by NH2-SiO2/Ru $(bpy)_3^{2+}$-UiO66 based Solid-state ECL.Electroanalysis. 2020;32:462-469. DOI:10.1002/elan.201900424

[45] Y.J. Muhammad Saqib, Shahida Bashir b, Shimeles Addisu Kitte, Haijuan Li: High-efficiency cathodic electrochemiluminescence of tris(2,2′- bipyridine)ruthenium(II)/N-hydroxy compounds system and its use for sensitive "turn-on" detection of mercury (II) and methyl blue. Chem. Commun. 2020;56:1827-1830. DOI:10.1039/C9CC09973E

[46] J. Yan, L. Ge, X. Song, M. Yan, S. Ge, J. Yu: Paper-Based Electrochemiluminescent 3D Immunodevice for Lab-on-Paper, Specific, and Sensitive Point-of-Care Testing. Chem. Eur. J.2012;18:4938-4945. DOI:10.1002/chem.201102855

[47] S.C. Somasekhar R. Chinnadayyala, Jinsoo Park, Hien Thi Ngoc Le, Mallesh Santhosh, Abhijit N. Kadam: Recent Advances in Microfluidic Paper-based Electrochemiluminescence Analytical Devices for Point-of-care Testing Applications. Biosens. Bioelectron. 2019;126:68-81. DOI:10.1016/j.bios.2018.10.038